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Full length report

Cannabidiol attenuates haloperidol-induced catalepsy and c-Fos protein expression in the dorsolateral striatum via 5-HT$_{1A}$ receptors in mice

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Highlights

- Cannabidiol (CBD) attenuated haloperidol-induced catalepsy.
- CBD reduced c-Fos protein expression in the dorsal striatum induced by haloperidol.
- CBD effects were blocked by 5-HT_{1A} receptor antagonist.

Abbreviations:

CBD - cannabidiol

THC - Δ^9-tetrahydrocannabinol

Abstract

Cannabidiol (CBD) is a major non-psychoactive compound from *Cannabis sativa* plant. Given that CBD reduces psychotic symptoms without inducing extrapyramidal motor side-effects in animal models and schizophrenia patients, it has been proposed to act as an atypical antipsychotic. In addition, CBD reduced catalepsy induced by drugs with distinct pharmacological mechanisms, including the typical antipsychotic haloperidol. To further investigate this latter effect, we tested whether CBD (15-60mg/kg) would attenuate the catalepsy and c-Fos protein expression in the dorsal striatum induced by haloperidol (0.6mg/kg). We also evaluated if these effects occur through the facilitation of 5-HT_{1A} receptor-mediated neurotransmission. For this, male *Swiss* mice were treated with CBD and haloperidol systemically and then subjected to the catalepsy test. Independent groups of animals were also treated with the 5-HT_{1A} receptor antagonist WAY100635 (0.1mg/kg). As expected, haloperidol induced catalepsy throughout the experiments, an effect that was prevented by systemic CBD treatment 30 minutes before haloperidol administration. Also, CBD, administered 2.5 hours after haloperidol, reversed haloperidol-induced catalepsy. Haloperidol also increased c-Fos protein expression in the dorsolateral striatum, an effect attenuated by previous CBD administration. CBD effects on catalepsy and c-Fos protein expression induced by haloperidol were blocked by the 5-HT_{1A} receptor antagonist. We also evaluated the effects of CBD (60nmol) injection into the dorsal striatum on haloperidol-induced catalepsy. Similar to systemic administration, this treatment reduced catalepsy induced by haloperidol. Altogether, these results suggest that CBD acts in the dorsal striatum to improve haloperidol-induced catalepsy via postsynaptic 5-HT_{1A} receptors.

Keywords: cannabinoids, catalepsy, typical antipsychotics, Fos expression, 5-HT_{1A} receptors.

1. Introduction

Antipsychotic drugs are used for the treatment of schizophrenia and act mainly as antagonists of dopamine D_2 receptors. They can be classified into two major groups, typical and atypical. Typical antipsychotics comprise older agents that are effective in attenuation of positive symptoms (e.g., delusions, hallucinations) but can induce several adverse effects (e.g. Parkinson-like symptoms) due to their high affinity for D_2 receptors. Atypical compounds include those with different pharmacological profile that are associated with a lower incidence of motor side effects [1].

The main adverse consequence of typical antipsychotics use is the extrapyramidal effects that follow the blockade of D_2 receptors in the nigrostriatal pathway. They include parkinsonian-like symptoms such as postural rigidity, slowness of movement and tremors [1]. In rodents these side effects are expressed as catalepsy, which is characterized by the maintenance of abnormal postures [2]. In fact, haloperidol, a typical antipsychotic, induces catalepsy in rodents, while clozapine, an atypical antipsychotic, does not [2,3]. This haloperidol effect is associated with neuronal activation of the dorsal striatum, indicated by an increased expression of the c-Fos protein, an effect that does not occur after treatment with atypical antipsychotics [3,4].

The striatum is a subcortical structure that belongs to a set of interconnected nuclei named the basal ganglia. Its dorsal portion is closely associated with sensorimotor function [5]. The striatum influence on motor control depends on two functionally opposite pathways. Whereas the direct pathway promotes a disinhibition of target structures and facilitates movement, the indirect pathway leads to inhibition of the thalamus and cortex to suppress the movement. Motor disorders are often associated with an imbalance between these two pathways, and in Parkinson's disease this balance is shifted to the indirect pathway [6].

Cannabidiol (CBD) is a major component of *Cannabis sativa* that is devoid of the psychoactive effects of its main psychotropic compound, Δ^9-tetrahydrocannabinol (THC). On the contrary, CBD is able to antagonize the psychotomimetic effects induced by high doses of THC, which lead to the proposal that CBD could have antipsychotic properties [7].

Preclinical studies suggest that CBD has a pharmacological profile similar to atypical antipsychotics, reducing psychotic-like symptoms at doses unable to induce

catalepsy [8,9,10,11]. Similar to clozapine, CBD increased c-Fos protein expression in the *nucleus accumbens* but not in the dorsal striatum [9]. Furthermore, CBD ameliorated psychotic-like symptoms induced by L-dopa treatment of Parkinson's disease [12]. At the same time, results from this study indicated that CBD could also improve motor function. Finally, a recent clinical-trial revealed that CBD was effective in reducing psychotic symptoms in schizophrenia patients without causing motor side-effects [13].

Based on these pieces of evidence, we investigated if CBD could attenuate catalepsy, as well as c-Fos expression in the dorsolateral striatum, induced by haloperidol. Also, considering that among the several mechanisms involved in its pharmacological effects, CBD can facilitated 5-HT_{1A} receptor-mediated neurotransmission [14,15,16] and agonists of this serotonergic receptor subtype attenuate haloperidol-induced catalepsy in rodents [17,18,19,20], we also tested if CBD effects would be mediated by 5-HT_{1A} receptors.

2. Material and methods

2.1. Animals

Male *Swiss* mice with 6 weeks of age (from the colony of mice maintained by the Campus of the University of São Paulo-Ribeirão Preto) were used in the experiments. Animals were housed in groups of 5 mice/cage, with water and food *ad libitum*, in a temperature-controlled (24±1°C) room and under 12 h light cycle (lights on at 7 am). The procedures were conducted in conformity with the standards of Brazilian Council for Care and Use of Laboratory Animals (COBEA), which is in accordance with the international laws and policies, and were approved by the local Ethical Committee (protocol number: 056/2012).

2.2. Drugs

The following drugs were used: cannabidiol (CBD; THCPharm, Germany), haloperidol (D_2 receptor antagonist; Haldol®, Janssen-Cilag, Brazil) and WAY100635 (5-HT_{1A} receptor antagonist; Sigma, USA). CBD was diluted in 2% Tween 80 in sterile saline (vehicle) for systemic administration. Haloperidol and WAY100635 were diluted in sterile saline (vehicle). For intra-dorsal striatum treatment, CBD was diluted in grape seed oil (vehicle, [21]).

2.3. Experimental procedures

2.3.1. Catalepsy test

The catalepsy test consists of placing the animal in an unusual posture over a horizontal glass bar (diameter= 0.5 cm) elevated 4 cm from the floor and recording the time it remains in this position. The animals were placed in the apparatus so that only the forepaws stay on the bar. The time that both forepaws remained on the apparatus was measured by an experimenter that was blind to the treatment conditions, with a maximum time of 300s. Catalepsy was considered finished when at least one forepaw touched the floor or when the mouse climbed upon the bar [2, 22].

2.3.1.1. Experiment 1: Effect of CBD pretreatment in the catalepsy induced by haloperidol

Animals received two i.p. injections with an interval of 30 minutes between them. The first injection was of CBD (15, 30 or 60 mg/kg) or vehicle followed by haloperidol (0.6 mg/kg) or vehicle. The animals were placed in the apparatus and the catalepsy time was measured 1, 2 and 4 hours after haloperidol injection. The intervals between drug injections and testing and the doses employed were based on previous results from the literature [10,22,23].

2.3.1.2. Experiment 2: Reversal of haloperidol-induced catalepsy by CBD

Animals received an i.p. injection of haloperidol (0.6 mg/kg) or vehicle followed, 2.5 hours later, by CBD (30 or 60 mg/kg) or vehicle. Catalepsy was measured 1, 2, 3 and 4 hours after haloperidol injection.

2.3.1.3. Experiment 3: Effect of pretreatment with a 5-HT_{1A} receptor antagonist in the CBD effects on catalepsy and dorsolateral striatum c-Fos protein expression induced by haloperidol

Animals received an i.p. injection of WAY100635 (a 5-HT_{1A} receptor antagonist; 0.1 mg/kg) or vehicle followed, 30 min later, by CBD (30 or 60 mg/kg) or vehicle administration. After another 30 min, they received an i.p. injection of haloperidol (0.6 mg/kg) or vehicle. Catalepsy was measured 2 hours after haloperidol injection.

Additionally, we also evaluated the effects of CBD on increased c-Fos protein expression in the dorsolateral striatum induced by haloperidol. Immediately after the catalepsy test, the animals were deeply anesthetized with urethane (25%, 5 mL/kg; ip) and transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA 4%) in 0.2 M phosphate buffer (PB). Then, the brains were removed and post-fixed in the same fixative solution (PFA 4%) for 2 h. After that, they were immersed in 30% sucrose in 0.01 M PBS for cryoprotection during 24 h. The brains were frozen at −40ºC on dry ice and isopentane and kept at -80°C until used. For c-Fos immunohistochemistry, 40 µm-thick serial coronal sections were obtained using a cryostat at -20ºC (CM-1900, Leica, Germany). The free-floating sections were rinsed (3 times, 5 min each) on PBS 0.1 M + 0.15% Triton-X (pH 7.4; washing buffer) and then pre-incubated for 30 min with 1% hydrogen peroxide in PBS to remove endogenous peroxidase activity. To avoid unspecific activity, free-floating sections were also incubated in a solution containing 5% bovine serum albumin (BSA), washing buffer and 5% normal goat serum for 1 h. Sections were incubated overnight with polyclonal rabbit anti-c-Fos antibody (1:1000, sc-52, Santa Cruz Biotechnology, USA). Subsequent to the primary antibody incubation, sections were successively washed (washing buffer) and incubated in a secondary antibody solution (PBS) for 1 h containing biotinylated goat anti-rabbit antibody (1:400; Vector Laboratories, USA). The sections were then incubated with the avidin-biotin immunoperoxidase method for 2 h (1:300, Vectastain ABC kit, Vector Lab, USA). c-Fos immunoreactivity was revealed by the addition of chromogen diaminobenzidine (Sigma-Aldrich, USA) into Tris-buffered saline and H_2O_2 0.02 %. All the reactions were performed at the temperature of 21ºC.

The slides were then mounted and visualized using a light microscope (Olympus BX50, Japan), coupled to a camera (Olympus DP72, Japan). Neurons were considered positive for c-Fos when they had brown spots within neuronal nuclei. The number of c-Fos-positive neurons in the dorsolateral striatum was counted by an observer that was blind to the treatment conditions using the image analysis software Image Pro-Plus 6.0 (MediaCybernetics, USA), which considered positive dark objects with an area between 10 and 80μm^2 [9].

2.3.1.4. Experiment 4: Intracerebral effect of CBD in the catalepsy induced by haloperidol

The animals underwent stereotaxic surgery to bilaterally implant cannulae (11 mm; diameter= 0.6mm) into the dorsal striatum (coordinates: anteroposterior= 1mm; lateral= ± 2mm; depth= 3mm; angle= 0°; [24]) fixed to the skull with acrylic cement. The surgeries were performed using a combination of ketamine (100 mg/kg) and xylazine (14 mg/kg) as an anesthetic and, immediately after the surgical procedure, the animals received the antibiotic Amoxicilin (5 g/L, administered in drinking water) for 7 days to prevent infection and post-surgical recovery.

After the recovery period, the animals received bilateral injections of CBD (60 nmol) or vehicle into dorsal striatum followed by systemic administration of haloperidol (0.6 mg/kg) or vehicle, with an interval of 5 minutes between the injections.

For the intra-dorsal striatum injections, needles (11.5 mm; diameter= 0.3 mm) connected to a syringe (10 μL; Hamilton, USA) through a segment of polyethylene (P10) were inserted into the guide cannulae. CBD or vehicle were administered with the help of an infusion pump (KD Scientific, USA) in a volume of 0.2 μL during 30 seconds. After the injections, the needles remained in the cannulae for an additional time of 30 seconds to prevent drug reflux. Catalepsy was measured 15, 30, 60 and 120 minutes after the haloperidol injection.

2.4. Statistical analysis

Data were analyzed by factorial ANOVA and, in case of interaction among the variables, one-way ANOVA was performed. The Student-Newman-Keuls (SNK) test was used for post-hoc comparisons. The non-parametric Kruskal-Wallis analysis followed by Mann-Whitney test was performed in the cases where there was no homogeneity of variance. The Pearson test was used to evaluate correlation between catalepsy time and c-Fos protein expression. Statistical results with $p<0.05$ were considered significant.

3. Results

3.1. Experiment 1: Effect of CBD pretreatment in the catalepsy induced by haloperidol

There were significant effects of time ($F_{2,98}=29.4$; $p<0.05$), treatment ($F_{5,49}=14.1$; $p<0.05$) and an interaction between time and treatment ($F_{10,98}=2.3$; $p<0.05$). Haloperidol induced catalepsy throughout the experiment (1h: $F_{5,49}=19$; 2h: $F_{5,49}=9.3$;

4h: $F_{5,49}$=7.8; SNK, p<0.05; Fig.1). All tested doses of CBD reduced the catalepsy induced by haloperidol when evaluated 1 ($F_{5,49}$=19; SNK p<0.05) and 2 hours ($F_{5,49}$=9.3; SNK, p<0.05) after administration of the typical antipsychotic, but not after 4 hours (SNK, p>0.05; Fig.1). As expected, CBD by itself did not induce catalepsy (SNK, p>0.05).

3.2. Experiment 2: Reversal of haloperidol-induced catalepsy by CBD

There were significant effects of time ($F_{3,72}$=9.7; p<0.05), treatment ($F_{4,24}$=14.1; p<0.05) and an interaction between time and treatment ($F_{12,72}$=1.9; p<0.05). Haloperidol induced catalepsy throughout the experiment (1h: $F_{4,24}$=7.8; 2h: $F_{4,24}$=7.3; 3h: $F_{4,24}$=11.3; 4h: $F_{4,24}$=4.6; SNK, p<0.05; Fig.2). CBD by itself did not produce catalepsy (SNK, p>0.05) but, at the dose of 60 mg/kg CBD attenuated haloperidol-induced catalepsy 30 minutes after haloperidol ($F_{4,24}$=11.3; SNK, p<0.05; Fig.2).

3.3. Experiment 3: Effect of pretreatment with a 5-HT_{1A} receptor antagonist in the CBD effects on catalepsy and dorsolateral striatum c-Fos protein expression induced by haloperidol

Haloperidol-induced catalepsy: As expected haloperidol induced catalepsy (χ^2=23.2; DF=5; p<0.05, Mann-Whitney, p<0.05; Fig.3). The 5-HT_{1A} receptor antagonist WAY100635 did not change the haloperidol-induced catalepsy (χ^2=25.8; DF=5; p<0.05, Mann-Whitney, p<0.05; Fig.3). As observed in the experiment 1, haloperidol-induced catalepsy was attenuated by CBD at the doses of 30 and 60 mg/kg (Mann-Whitney, p<0.05; Fig.3). WAY100635 did not induce catalepsy by itself (Mann-Whitney, p>0.05) but prevented the anti-cataleptic effects induced by the two tested doses of CBD (Mann-Whitney, p<0.05; Fig.3).

c-Fos immunohistochemistry: Haloperidol also increased the expression of c-Fos protein in the dorsolateral striatum (χ^2=29.9; DF=5; p<0.05, Mann-Whitney, p<0.05; Fig.4), an effect attenuated by CBD at the dose of 30 mg/kg (Mann-Whitney, p<0.05; Fig.4), but not at the dose of 60 mg/kg (Mann-Whitney, p>0.05). Pretreatment with WAY100635 did not changed the haloperidol effects (χ^2=27.4; DF=5; p<0.05, Mann-Whitney, p<0.05; Fig.4), but it blocked the effect produced by CBD on c-Fos protein expression induced by haloperidol (Mann-Whitney, p<0.05; Fig. 4). CBD and the 5-HT_{1A} receptor antagonist did not change c-Fos protein expression compared to controls

(Mann-Whitney, $p>0.05$). There was a significant correlation between the duration of catalepsy and c-Fos-protein expression in the dorsolateral striatum of the animals ($r=0.4$; $p<0.05$), indicating that the longer the duration of catalepsy the greater the c-Fos protein expression in the striatum.

3.4. Experiment 4: Intracerebral effect of CBD in the catalepsy induced by haloperidol

There were effects of treatment 1 (CBD or vehicle; $F_{1,20}=13.1$; $p<0.05$), treatment 2 (haloperidol or vehicle; $F_{1,20}=85.5$; $p<0.05$) and interaction between them ($F_{1,20}=14.3$; $p<0.05$). Haloperidol induced catalepsy throughout the experiment (15min: $F_{3,20}=21.8$; 30min: $F_{3,20}=59.6$; 60min: $F_{3,20}=29.7$; 120min: $F_{3,20}=18.4$; SNK, $p<0.05$), an effect attenuated by intra-striatal administration of CBD (SNK, $p<0.05$; Fig.5). CBD by itself did not promote catalepsy (SNK, $p>0.05$).

4. Discussion

The present study showed that both systemic and intra-striatal CBD administration attenuates and even reverses catalepsy induced by the typical antipsychotic haloperidol. CBD also reduced the increased c-Fos protein expression in the dorsolateral striatum induced by haloperidol. Moreover, the 5-HT_{1A} receptor antagonist WAY100635 prevented the behavioral and cellular effects of CBD.

The Parkinson-like symptoms in humans and catalepsy in rodents induced by haloperidol and the other typical antipsychotics depend on the degree of dopamine D_2 receptors occupancy in the striatum. In schizophrenia patients, these effects appear with a mean D_2 receptors occupancy of 82% [25]. Meanwhile, the atypical antipsychotic clozapine, which does not induce extrapyramidal effects at clinical doses, produces a D_2 receptor occupancy ranging from 38% to 63% [25].

Consistent with previous studies CBD, by itself, did not induce catalepsy. Indeed Zuardi *et al.* [8] showed that CBD, even at doses as high as 480 mg/kg, did not induce catalepsy in rats. Additionally, in a clinical study with patients with Parkinson's disease that presented L-dopa-induced psychotic symptoms, CBD reduced scores for positive psychotic symptoms without causing motor impairment. On the contrary, CBD

decreased the scores of all Unified Parkinson's Disease Rating Scale items, but not reaching statistical significance, including the item 'motor', although the total score of this scale decreased significantly [12]. More recently, in a double-blind study, CBD was as effective as amisulpride to decrease psychotic symptoms in schizophrenia patients without causing extrapyramidal side-effects [13].

In the present study CBD was able to prevent catalepsy promoted by haloperidol. This effect replicates a previous study from our group [22], in which CBD also decreased catalepsy induced by drugs that do not directly antagonize dopamine receptors, such as the nitric oxide synthase inhibitor L-NOARG and by the mixed synthetic $CB_{1/2}$ receptor agonist WIN55,212-2,. Moreover, chronic administration of a *Cannabis sativa* extract (18 days) had already been shown to reduce haloperidol-induced catalepsy. This effect appeared on the first day of treatment, persisting until the fifteenth day [26]. Since Δ^9-THC induces catalepsy in mice [27] and enhances haloperidol effects [28], it is possible suggesting that CBD could be responsible for the effect observed with this *Cannabis* extract.

When CBD was administered after haloperidol, CBD was also able to decrease catalepsy induced by this antipsychotic. This effect was similar to that induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT, which completely antagonize catalepsy promoted by haloperidol when 8-OH-DPAT was administered 2.5h after haloperidol [29]. Other studies have also indicated that prior administration of a 5-HT_{1A} receptor agonist attenuates haloperidol-induced catalepsy, an effect blocked by the 5-HT_{1A} receptor antagonist WAY100635 [17,18,19,20]. Similarly, Gomes *et al.* [22] demonstrated that WAY100635 blocked the anticataleptic CBD effect over not only haloperidol but also a nitric oxide synthase inhibitor and a $CB_{1/2}$ receptor agonist. Corroborating these results, we also observed that CBD effects on catalepsy and increased c-Fos protein expression in the dorsolateral striatum induced by haloperidol were blocked by the pretreatment with WAY100635.

The proto-oncogene *c-fos* can be rapidly and transiently induced in the central nervous system after physiological, pharmacological, electrical and surgical stimuli, and its expression is correlated to the intensity of the increase in neuronal activity [30]. c-Fos protein expression in the dorsal striatum by haloperidol has been related the ability of this drug to block striatal dopamine D_2 receptors and, consequently, induce extrapyramidal symptoms [4]. Similar to 5-HT_{1A} receptor agonist, we observed that CBD caused a specific reduction in haloperidol-induced c-Fos protein expression in the

dorsal striatum [20]. Interestingly, we observed a positive correlation between catalepsy time and c-Fos protein expression in the dorsolateral striatum. And, as reported before, CBD, by itself, failed to increase c-Fos protein expression in this region [9]. In addition, it has been observed that facilitation of 5-HT_{1A} receptor-mediated neurotransmission attenuated the increase in the expression of transcription factors such as *Nur77* e *Nor-1* induced by acute administration of haloperidol [31].

Although the mechanisms of 5HT1A–mediated attenuation of haloperidol motor effects are not altogether clear, agonists of this receptor reduce glutamate and GABA levels in the striatum and substantia nigra, respectively, which have been associated with their antiparkinson effects [32]. Serotonin 5-HT_{1A} receptors are located mainly in the cell bodies and dendrites of serotonergic neurons in the raphe nuclei and post-synaptically in limbic structures (for review see [33,34]). Autosomic 5-HT_{1A} receptors inhibit the activity of dorsal raphe neurons. In forebrain structures they also induce inhibitory effects by inhibiting adenylate cyclase (for review see [33]). Autosomal and/or post-synaptic 5-HT_{1A} receptors are proposed to modulate the dopaminergic system. Buspirone and 8-OH-DPAT, partial and full 5-HT_{1A} receptor agonists respectively, attenuate haloperidol-induced catalepsy [18,35]. Stimulation of autosomal 5-HT_{1A} receptors could release dopaminergic inhibition promoted by serotonin since activation of these receptors decreases the availability of serotonin in basal ganglia. On the other hand, stimulation of postsynaptic 5-HT_{1A} receptors seems to counterbalance the stimulatory effect due to blockade of striatal dopamine D_2 receptors [18].

Regarding CBD, however, our results point to a postsynaptic 5-HT_{1A} receptor effect, since the drug was also effective after direct administration into the dorsal striatum. Corroborating this finding, Mignon and Wolf [36] showed that 8-OH-DPAT attenuated the hypolocomotor activity of rats with reserpine-induced monoamine depletion, an effect that was blocked by 5-HT_{1A} receptor antagonist WAY100635. Therefore, the motor effects of 5-HT_{1A} receptor agonists are maintained even after a drastic reduction of serotonin levels, suggesting the involvement of postsynaptic receptors [34, 36]. Even if other regions such as the motor cortex could be also involved in the anticataleptic effects of 5-HT_{1A} receptor agonists [37,38], similar to our results, bilateral microinjections of 8-OH-DPAT into the dorsolateral striatum also reduced, via 5-HT_{1A} receptors, catalepsy induced by haloperidol [38].

The mechanisms in which CBD facilitates the 5HT_{1A} receptor-mediated neurotransmission are still poorly understood. The drug was originally described to act

as an agonist at these receptors. Russo *et al.* [14] reported that CBD displaced 8-OH-DPAT radioactively marked from cloned human 5-HT_{1A} receptors expressed in Chinese hamster ovary cultured cells and increased the binding of labeled GTP at these receptors. More recent work, however, showed that CBD potentiated 8-OH-DPAT-induced effects *in vitro* and it may involve interactions with allosteric sites of 5-HT_{1A} receptors [39]. Moreover, our results agree with a wealth of evidence indicating that the anxiolytic [15], antiemetic [39] and neuroprotective [40] effects of CBD depend on facilitation of 5-HT_{1A}-mediated neurotransmission (for review see [16]).

In conclusion, the blockade of D_2 receptors by haloperidol in the nigrostriatal pathway results in the appearance of extrapyramidal side effects, here measured by catalepsy. CBD, by a still incompletely understood mechanism, facilitates 5-HT_{1A} receptor-meditated neurotransmission in the striatum and relieves haloperidol-induced extrapyramidal symptoms. The results, therefore, suggest that this phytocannabinoid has therapeutic potential for the treatment of extrapyramidal side effects caused by typical antipsychotics, as well as other striatal disorders.

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Figures

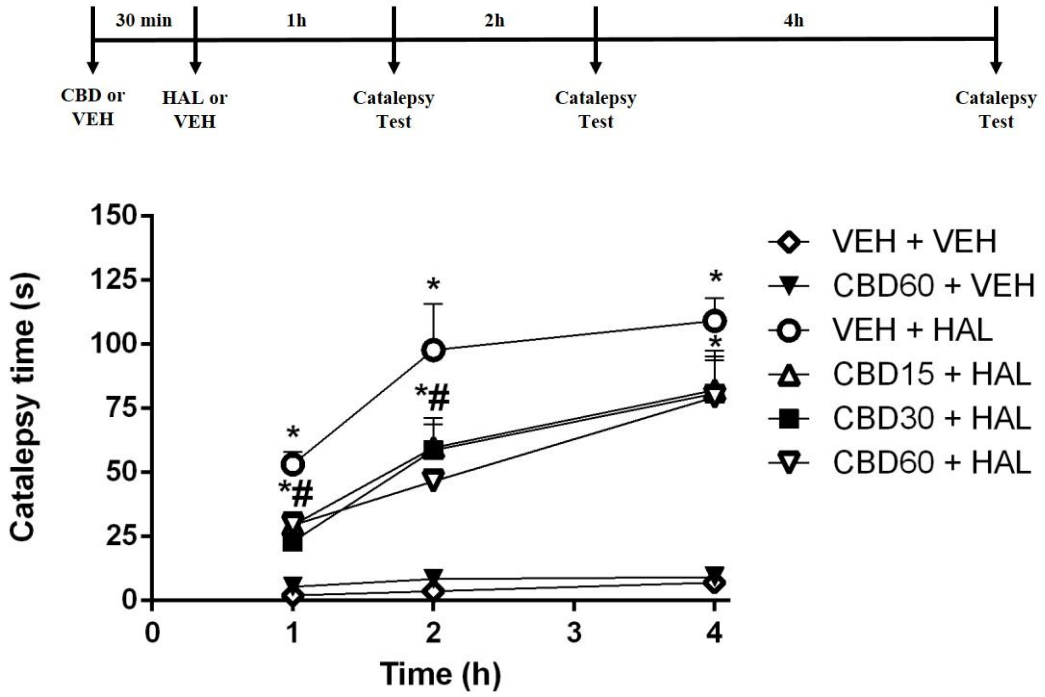


Figure 1 – Effect of CBD (15, 30 and 60 mg/kg) or vehicle (VEH) on catalepsy induced by haloperidol (HAL, 0.6 mg/kg). CBD prevented the haloperidol-induced catalepsy time evaluated 1 and 2h after haloperidol administration. Data presented as mean ± SEM (n=6-12 animals/group). * $p<0.05$ from VEH+VEH group and # $p<0.05$ from VEH+HAL, SNK test.

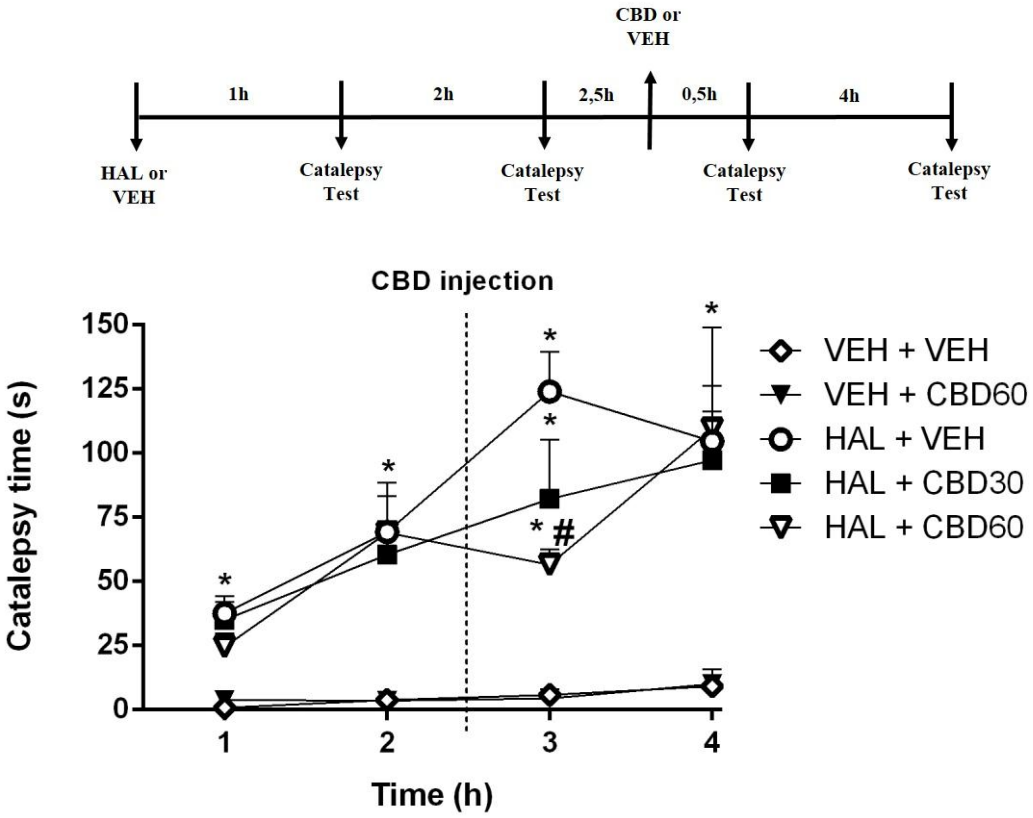


Figure 2 – Effect of post-treatment with CBD (30 and 60 mg/kg) or vehicle (VEH) on catalepsy induced by haloperidol (HAL 0.6 mg/kg). CBD, administered 2.5h after haloperidol, decreased the catalepsy time when evaluated 30min after its administration. Data presented as mean ± SEM (n=5-7 animals/group). * $p<0.05$ from VEH+VEH group and # $p<0.05$ from HAL+VEH, SNK test

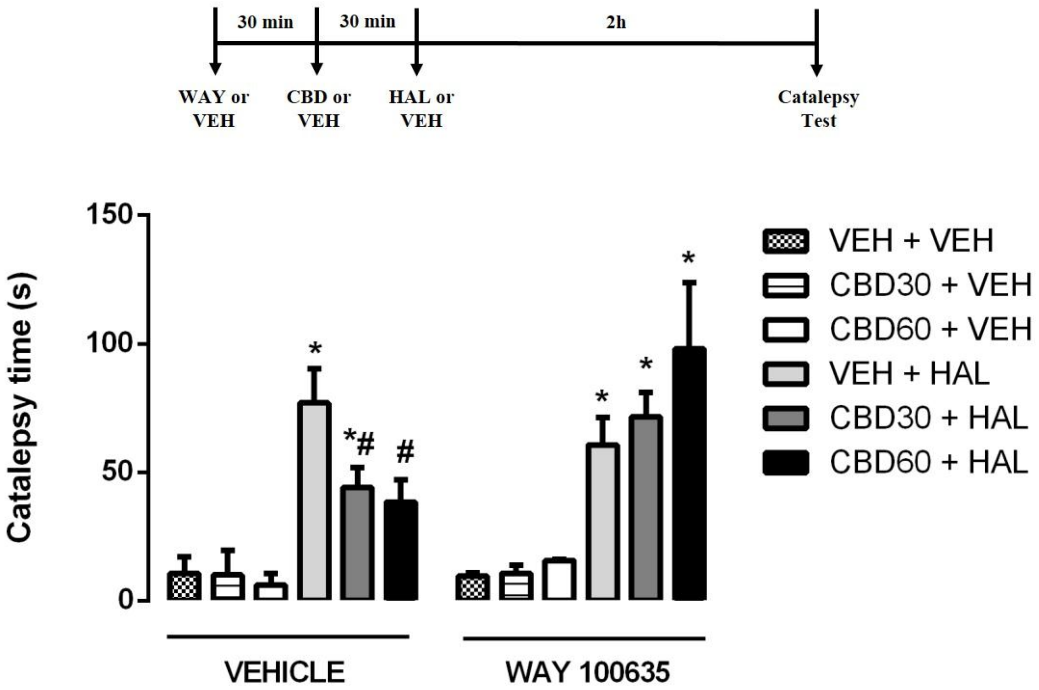
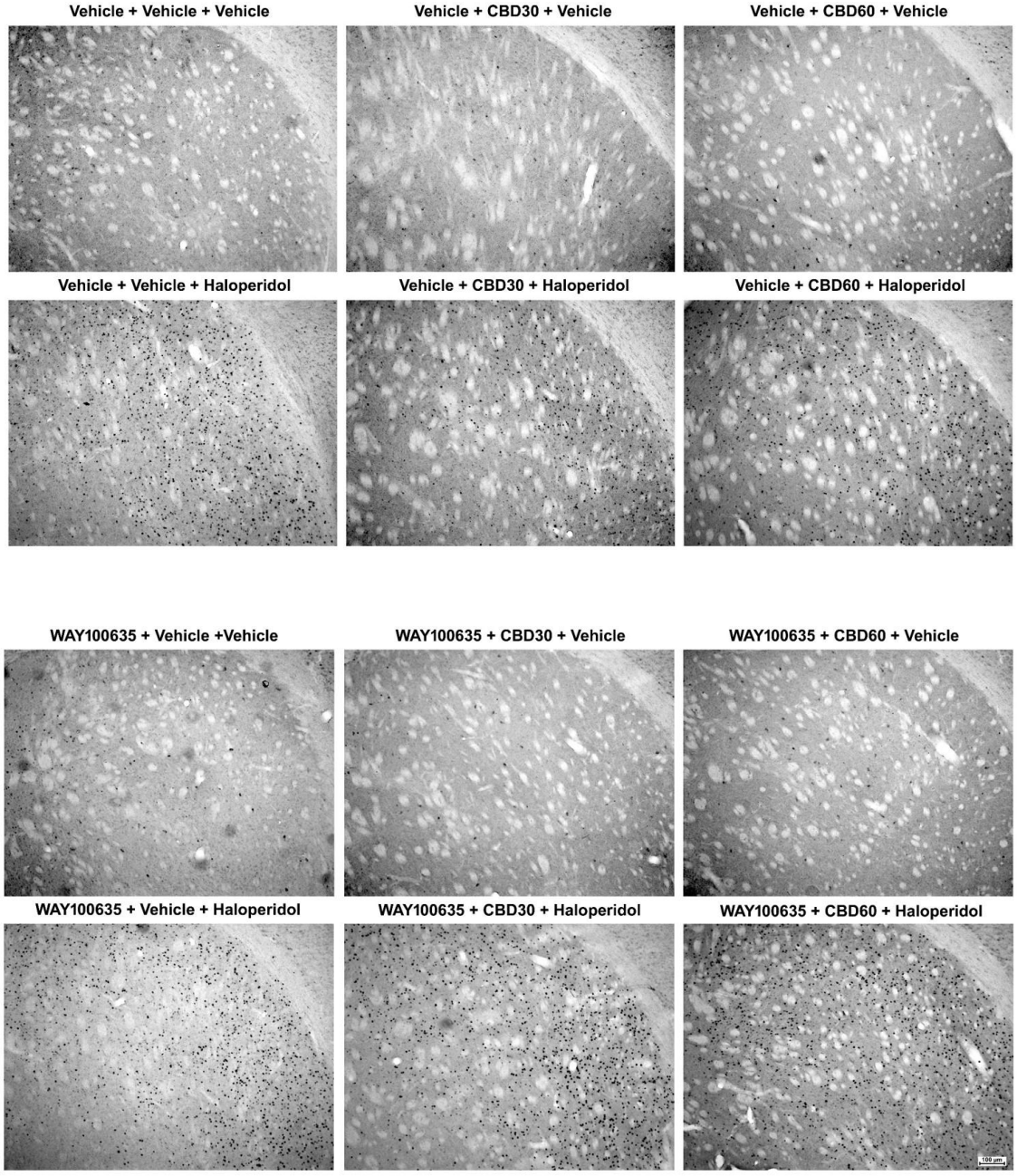


Figure 3 – Effect of pretreatment with WAY100635, a 5-HT_{1A} receptor antagonist (0.1 mg/kg), or vehicle on anti-cataleptic effect of CBD (30-60mg/kg). Anticataleptic effects of CBD were blocked by WAY10035. Data presented as mean ± SEM (n=3-8 animals/group). * $p<0.05$ from VEH+VEH+VEH and # $p<0.05$ from VEH+VEH+HAL, Mann-Whitney test. After the evaluation of catalepsy time, the brain of the animals was removed and processed for c-Fosprotein immunohistochemistry (see Fig. 4).



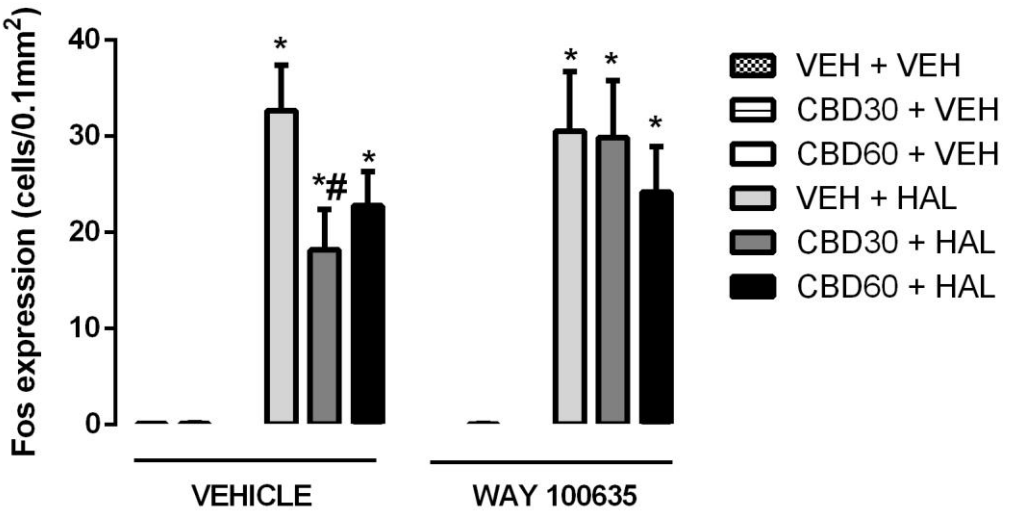


Figure 4 – Effect of pretreatment with WAY100635 (0.1 mg/kg) or vehicle followed by a second injection of CBD (30-60 mg/kg) or vehicle (VEH) on c-Fos expression induced by haloperidol (HAL, 0.6 mg/kg). CBD decreased the expression of c-Fos protein in the dorsal striatum induced by haloperidol, an effect blocked by the 5-HT_{1A} receptor antagonist WAY100635. Data presented as mean ± SEM (n=3-8 animals/group).* $p<0.05$ from VEH+VEH+VEH and # $p<0.05$ from VEH+VEH+HAL, Mann-Whitney test.

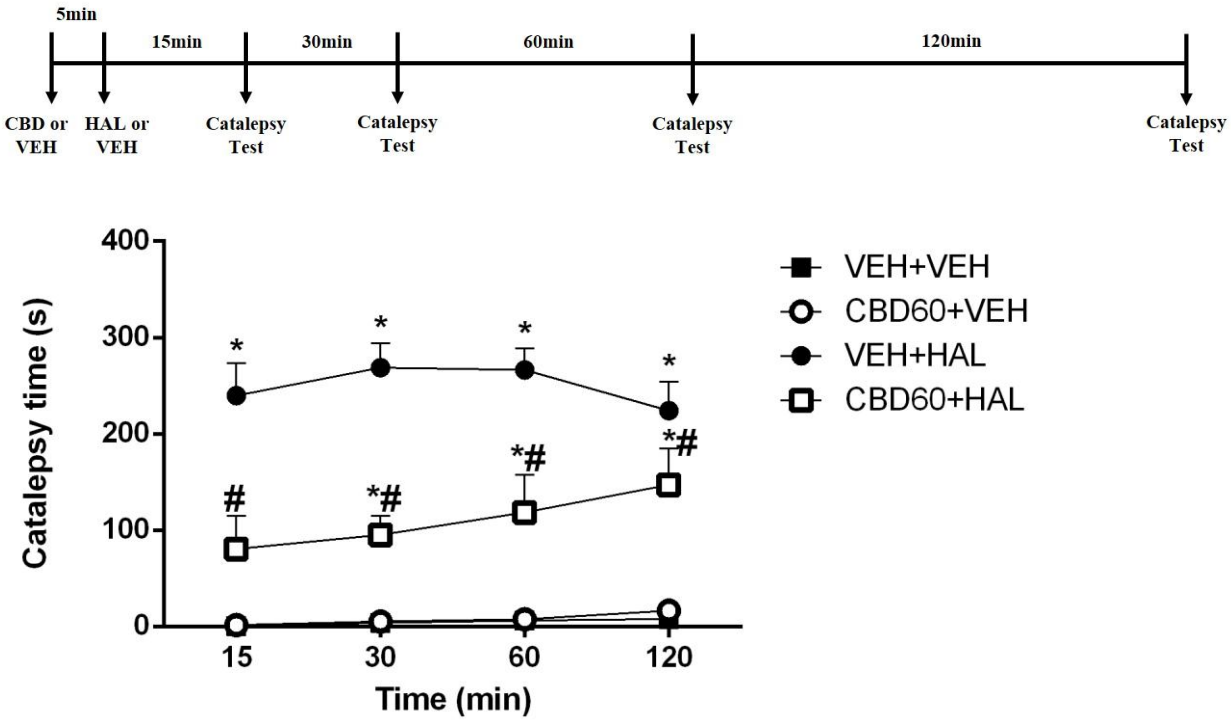


Figure 5 – Effect of intra-striatal CBD (60 nmol) or vehicle (VEH) on catalepsy induced by systemic haloperidol (HAL, 0.6 mg/kg). CBD infused into dorsal striatum attenuated haloperidol-induced catalepsy. Data presented as mean ± SEM (n=6 animals/group). * $p<0.05$ from VEH+VEH and # $p<0.05$ from VEH+HAL, SNK test.